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(54) Title: LEPTIN ASSAY

(57) Abstract

A trans-activation assay for leptin is described. Leptin response elements are located proximal to a promoter, and the promoter region is operatively linked to a reporter gene. When leptin binds with the receptor, the reporter gene is transcribed.

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TITLE OF THE INVENTION

LEPTIN ASSAY

FIELD OF THE INVENTION

This invention relates to an assay for determining the presence of compounds which bind to a leptin receptor. This invention also relates to an assay for quantifying the amount of a leptin receptor binding agent present in a sample.

BACKGROUND OF THE INVENTION

Leptin is a product of the Ob gene (Zhang *et al.*, 1995 *Nature* 372:425-432). In its absence, mice develop extreme obesity resembling morbid obesity of humans. They also develop diabetes resembling human type II diabetes (Zhang *et al.*, 1995 *Nature* 372:425-432), and become infertile (Chehab *et al.*, 1996 *Nature Genetics* 12:318-320). Repletion of leptin leptin reverses these pathologies.

Currently, the only manner in which preparations of recombinant leptin can be assayed is by injection of large quantities into *ob/ob* mice (Campfield 1995 *Science* 269:546-549; Rentsch 1995 *Biochem Biophys Res Comm* 214:131-136). Leptin activity is assessed based upon depression of feed intake and is highly variable among individuals. No mutually agreed upon standard of leptin activity exists. Therefore, a rapid, accurate assay for leptin bioactivity which utilizes small amounts of leptin would be desirable.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a method for determining if a leptin receptor binding compound is present in a sample comprising:

contacting the sample with a cell which comprises a) nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor; and

determining if transcription of the reporter gene occurs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing functional assay of leptin using GT1-7 cells. Cells were either: mock transfected with respect to ob-r, transfected with lean rat Ob-r cDNA, or transfected human Ob-r cDNA and showed induction of luciferase activity in cell expressing Ob-r treated with leptin.

Figure 2 is a graph showing that cells respond in a linear, dose-responsive manner to the addition of leptin as determined by increase in luciferase activity. GT1-7 cells were transiently transfected with human Ob-r and treated with various amounts of human leptin.

Figure 3 is the DNA sequence for lean rat Ob-r.

Figure 4 is the DNA sequence for human Ob-r which has been placed in the pCMV4 vector. The Gen Bank accession number for human ob-r is U43168. Nucleotides 141-37-7-0 are used in pCMV4-Ob-4.

Figure 5 is the DNA sequence for *fatty* rat Ob-r.

As used throughout the specification and claims, the following definition shall apply:

"Leptin receptor binding compound" means a compound which binds to a leptin receptor.

"Leptin mimetic" means a compound other than leptin which binds to a leptin receptor and triggers a cascade of intracellular reactions which ultimately results in transcription of DNA under the control of leptin response elements.

"Leptin response elements" means DNA sequences located within a promoter region which are responsive to a leptin receptor binding event, and in the presence of such an event, allow for transcription of the DNA under the control of the promoter.

"Promoter region" means DNA located upstream of a protein or peptide encoding sequence, and includes at least one response element.

"Promoter" includes full length promoters, minimal promoters and promoters which are less than full length, but include more nucleic acids than minimal promoters.

"Ob-r" means Ob-receptor.

One aspect of this invention is a cellular "trans" activation assay which can determine if a particular compound can bind to a leptin receptor. In general, the binding of a ligand to its membrane-bound receptor initiates an intercellular cascade of signals. Ultimately, a specific transcriptional control element is activated, leading to the transcription of DNA. It has been found, in accordance with this invention, that the binding of native leptin with its native leptin receptor initiates a cascade which activates leptin response elements, leading to the transcription of DNA. One such leptin response element which has been identified in accordance with this invention is an IRF-1 derived gamma-interferon activation sequence. IRF-1 derived activation sequences have been described in the art (Pine *et al.*, 1994 *EMBO J.* 13:158-167) but their responsiveness to leptin-leptin receptor binding events has been heretofore unknown.

This discovery of a leptin response element has allowed for the design of various assays which form the basis of this invention. A promoter region is constructed which contains a promoter of choice and one or more leptin response elements. The promoter region may contain be a hybrid promoter region, i.e. contain a promoter and response elements which do not naturally occur together, or they may be a naturally occurring promoter region. In a preferred embodiment the promoter is a well-characterized promoter, such as the herpes simplex virus thymidine kinase promoter, but any promoter which is known to function in the host cell chosen may be used. It is also preferred that the promoter be a minimal promoter, so that there are no transcriptional control sequences which may influence the activity of the leptin response element(s).

The leptin-response element is preferably placed proximal to the promoter region. Intervening sequences may be present between the leptin response element and the promoter, provided they do not interfere with the functioning of the leptin response element.

In accordance with this invention, a suitable leptin response element is an IRF-1 derived gamma interferon activation sequence

5'-CTGATTTCCC CGAAATGACG-3'. In a preferred embodiment, the promoter region comprises a plurality of IRF-1 derived gamma interferon activation sequences; and more preferably it comprises at least three such sequences. When a plurality of leptin response elements are present, they are preferably joined in tandem. However, there may be interviewing sequences present provided they do not interfere with the functioning of the leptin response elements.

In order to detect transcription which occurs as a result of the leptin-leptin receptor binding, it is preferred to operatively link a reporter gene to the promoter region which contains at least one leptin response element. A reporter gene may be any gene which encodes a peptide which is easily detected, or otherwise allows for easy detection of transcription or translation. It generally encodes a protein which does naturally occur in the host cell or only is produced in small amounts by the host cell. Examples of well known reporter genes include: chloramphenicol acetyl transferase (CAT), green fluorescent protein (GFP), luciferase (either bacterial or firefly), and other enzyme-based detection systems such as β-galactosidase, alkaline phosphatase, and the like. In a particularly preferred embodiment, luciferase is a reporter gene. In alternative embodiments, the mRNA transcribed from the reporter gene DNA may be measured rather than the translation product.

The reporter gene construct, which comprises a) the promoter region which contains a promoter and at least one leptin response element and b) the reporter gene operatively linked to the promoter region forms yet another aspect of this invention. It is preferably placed in an appropriate vector and in used to transfect a host cell. This vector comprises yet another aspect of this invention. The vector may be any known vector, including plasmids, cosmids and viral vectors which can function in a chosen host cell.

The host cell may be any cell or cell line which is conveniently cultured. In general, a preferred host cell will be eukaryotic, preferably mammalian, and in particularly preferred embodiments it is a mouse hypothalamic cell (such as GT1-7), a

mammalian neuroblastoma cell line, mouse fibroblast cell lines such as NIH/3T3 (ATCC CRL 1658) or L (ATCC CCL 1), Chinese hamster ovary CHO-K1 cells (ATCC CCL 61) or human embryonic kidney-derived 293 cells (ATCC CRL 1573).

The host cell should either have an abundance of leptin receptors on its surface or it can be transformed to express an abundance of leptin receptors on its surface, the latter situation being the preferred one. Any leptin receptor may be used; human and rodent ones are preferred. Nucleic acids encoding human leptin receptors have been described (Tartaglia *et al.*, 1995 *Cell* 83:1263-1271 which is hereby incorporated by reference) and are shown in Figure 4. Mouse genes, can also be used, especially the position 26 to 2775, based on the sequence given in Gen Bank. Nucleic acids encoding leptin receptors from wild-type rats, *fatty* rats (*falfa* genotype) (shown in Figure 3) and rats heterozygous for the *ob* receptor gene (shown in Figure 5) are described in co-pending U.S. application Serial Nos. ____ and 60/013,969, Attorney Docket Nos. 19462PV filed February 22, 1996 and 19462PV2, filed March 22, 1996, both of which are hereby incorporated by reference. By transfecting cells with various leptin receptors, the biological response of receptors from different sources can be studied and compared. Also, leptin receptors containing particular desired mutations can be constructed according to known molecular biological techniques and the structure and function of the mutants can be studies using the assay of this invention.

Another aspect of this invention is a set of vectors suitable for transfecting a host cell so that it can be used in the assays of this invention. The set of vectors comprises a first vector which contains a leptin receptor construct. The leptin receptor construct includes nucleic acids encoding a desired leptin receptor or mutated form of the receptor. It may be under the control of its native promoter or any other desired heterologous promoter. Optionally it may also contain other expression-control elements, such as enhancers and sequences which assist in expressing the receptor on the membrane.

The set of vectors also comprises a second vector comprising the receptor gene construct described previously.

In a preferred assay of this invention, compounds which are suspected of being leptin receptor ligands can be assayed. In this embodiment, cells which express a leptin receptor (either natural or recombinant) are transfected with the reporter gene construct described previously and is transfected with a leptin receptor construct, if the cell does not naturally express an abundance of the leptin receptor. The putative leptin receptor ligand is placed in contact with the transfected cells, and the presence of the reporter gene transcription or translation product is detected. This may be compared to the amount of transcription or translation measured in a control assay, where an identically transfected cell is placed in contact with leptin. A further advantage of the assay of this invention is that it is dose-responsive; i.e. as more leptin ligand-leptin receptor binding occurs, transcription and/or translation of reporter gene increases, and therefore allows for a quantitative determination of leptin receptor binding activity.

A counterscreen may also be employed as a part of this assay. In the counterscreen, a second cell of the same cell type is transfected with the reporter gene construct described previously, but is not transfected with the leptin receptor gene construct. The putative leptin receptor ligand is placed in contact with the transfected cells and the presence of of the reporter gene transcription or translation is detected. Those putative leptin receptor ligands which activate the reporter gene construct only in the presence of leptin receptor are determined to be specific leptin agonists.

Using this embodiment of the assay, leptin agonists and antagonists may be identified. A leptin agonist is a compound which binds to the leptin receptor, such as a leptin mimetic, and produces a cellular response which is at least about equivalent to that of leptin, and which may be greater than that of leptin. Such compounds would be useful in situations where leptin insufficiency causes obesity, diabetes or infertility.

Also using this embodiment of the assay, leptin antagonists may be identified. A leptin antagonist is a compound which can bind to the leptin receptor, but produces a lesser response than that of native leptin. Such compounds would be useful in the treatment of anorexia and cachexia.

In another aspect of this invention, novel leptin response elements can be identified by inserting various putative leptin response elements proximal to a promoter which controls the transcription of a reporter gene. A cell transfected with this construct and expressing natural or recombinant leptin receptors is contacted with leptin. The resulting transcription of the reporter gene is compared with that which occurs when the leptin response element is an IRF-1 derived gamma interferon activation sequence. Novel leptin response elements which are identified using this assay form yet another aspect of this invention.

In another embodiment of this invention, the potency of a leptin preparation can be determined, and quantified. In this embodiment, the leptin-containing preparation is contacted with the cell containing the reporter gene construct and which is expressing leptin receptors (either recombinant or native). The amount of transcription and/or translation of the reporter gene is measured and is compared to that obtained using preparations of known potency.

In yet another aspect of this invention, the biological activity of various leptin receptors can be studied and compared. In this embodiment, a first cell is transfected with a reporter gene construct and a first leptin receptor. The first leptin receptor can be any leptin receptor or variant or mutant whose activity is to be determined. The first cell is put into contact with leptin and the reporter gene transcription or translation is measured. This amount is compared to that obtained under the same assay conditions using a second leptin receptor whose activity is known or is otherwise to be used as a reference.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Vector Construction

AH32, an expression vector transactivated by leptin, was constructed as follows. Two complimentary oligonucleotides containing the Stat Binding Element (SBE) from the IRF-1 gene (Sims *et al.*, 1993 *Mol Cell Biol.* 13:690-702; and Pine *et al.*, 1994 *EMBO J.* 13:158-167; both of which are hereby incorporated by reference) were synthesized, kinased and annealed using standard molecular biology techniques (Sambrook *et al.*, 1989 *Molecular Cloning: A Laboratory Manual*, 2nd Ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). The sequences of the oligonucleotides used is 5'-CTGATTTCCC CGAATGACG-3' and 5'-CGTCATTTCG GGGAAATCAT-3'. The double stranded oligonucleotides were cloned into a *Sma I* site of pTKLuc. Plasmid pTKLuc contains a promoter sequence from -35 to +10 of the herpes simplex virus thymidine kinase gene (McKnight *et al.*, 1982 *Science*, 217:316-324) upstream of the firefly luciferase gene found in pZLuc (Schadlow, *et al.*, 1992 *Mol. Biol Cell* 3:941-951). The ligated DNA was used to transform *E. coli* using DH5α competent cells (Gibco/BRL, Gaithersburg, MD). DNA obtained from the transformed colonies was analyzed by restriction digest analysis and the orientation and number of copies of SBE oligonucleotides found in each of the transformants was confirmed by sequencing through the insert using a sequencing kit (US Biochemical, Cleveland, OH). Clone AH32 was found to contain 3 copies of the SBE inserted in the same orientation and was used for further studies with a human fibroblast cell line (WI-38 VA13 subline 2RA; ATCC No. CCL 75.1). Cells transfected with the AH32 showed a 6-10 fold increase in the luciferase activity after 6h of treatment with IFN-α or IFN-γ.

Primers ROBR 35 (5'-TTGGAGGACT ATGGGTGTC-3') and ROBR 37 (5'- CTACTGGAAT GGAACCTGG-3') were used to obtain the full length coding sequence for the rat OB-R, (approximately 3.7 kb in size) by the PCR using rat hypothalamic cDNA as the template. Long PCR produced the full length fragments by a modified method of Barnes (Barnes, 1994, *Proc. Natl. Acad. Sci.* 91:2216-2220,

which is hereby incorporated by reference). For the long PCR reactions, Taq Extender (Stratagene, La Jolla, CA) and the Expand Long Template PCR System (Boehringer Mannheim, Indianapolis, IN). were used in combination. The standard PCR reaction mix, in a final volume of 20 µl, consisted of 40 ng of template (rat cDNA), 200 ng of primers, 500 mM dNTPs, 1 X Buffer 3 from the Expand kit. and 0.2 ml of the Expand kit enzyme mix. Reactants were assembled in thin walled reaction tubes. The amplification protocol was 1 cycle of 92°C for 30 sec., followed by 35 cycles at 92°C for 30 sec., 54°C for 1 min. and 68°C for 5 min. using a Perkin Elmer 480 Thermal Cycler.

The DNA sequence of the full length products was determined using ABI PRISM Dye terminator cycle sequencing with AmpliTaq DNA polymerase, FS (Perkin Elmer, Foster City), and a number of PCR induced errors were detected. To eliminate these PCR induced mutations, restriction fragments from clones containing the correct DNA sequence were assembled. Three different restriction fragments were used to construct a full length clone derived from *fa/fa* hypothalamic cDNA, with a C at nucleotide 880. The expression construct was obtained by ligating a fragment from a hind III polylinker site to a *Nde* I site at nucleotide 502, a fragment from the *Nde* I site at 502 to a *Sac* I site at 1522, and a fragment from the *Sac* I site at 1522 to a *Xba* I polylinker site into the *Hind* III and *Xba* I sites of pcDNA 3 (Invitrogen, San Diego, CA). The wild type (lean) clone, with an A at nucleotide 880, was constructed by replacing the *Nde* I by *Sac* I fragment from 502 to 1522 with a fragment derived from lean hypothalamic cDNA.

pCH110, a vector expressing β-galactosidase under control of the SV40 early promoter (Hall *et al.*, 1983, *J Mol Appl Gen* 2:101-109), was obtained from Pharmacia Biotech, Inc. (Piscataway, NJ).

A fragment of the human Ob receptor cDNA, nucleotides 141-3770, containing the entire coding region was obtained using PCR and subcloned into pCMV4 (Andersson, S. 1989 *J Biol. Chem.* 264:8222-8229) a gift of D.W. Russell.

EXAMPLE 2

Cell Culture

GT1-7 cells, a mouse hypothalamic cell line, a gift of P. L. Mellon (Liposits *et al.*, 1991 *Endocrinology* 129:1575-1583) were maintained in Dulbecco-modified Eagle Medium (DMEM) (Gibco/BRL, Gaithersburg, MD), 10 % bovine calf serum, 50 µg/ml streptomycin, and 50 U/ml penicillin (growth medium) at 37°C in an atmosphere of 95% air, 5% CO_2. Cells were seeded at approximately 4x $10^4/cm^2$ and passaged before confluence.

EXAMPLE 3

Transfection of GT1-7 Cells

18 hours before transfection, cells were plated at 3.5 x 10^5 cells per 35 mm tissue culture dish. The plasmids AH32, appropriate Ob-r plasmid and pCH110 were introduced into cells using liposome-mediated transfection. Specifically, 300 ng of each plasmid was added to 200 µl of Optimem™ (Gibco/BRL, Gaithersburg, MD). 10 µg of Lipofectamine™ (Gibco/BRL, Gaithersburg, MD) was added to a second 200 µl volume of Optimem™. The two solutions were mixed, incubated at room temperature for 30 minutes and adjusted to 600 µl with Optimem™. Cells were washed twice with phosphate buffered saline. The DNA-liposome complex was applied to cells. Cells were incubated for 6 hours at which time 2 ml of growth medium was added to cells. 16 hours later, the transfection mixture was removed and 2 ml fresh growth medium was added.

EXAMPLE 4

Luciferase Assay

Approximately 24 hours after transfection, various amounts of recombinant human leptin were added to cells. Cells were then incubated for 24 hours. Cell culture medium was removed and cells were washed promptly with 2 ml of ice-cold calcium and magnesium-

free phosphate buffered saline. Cells were then prepared for assay with 150 µl Promega reporter lysis reagent (Promega, Madison, WI) according to the manufacturer's protocol. 20 µl of cell lysates was then assayed for luciferase activity using a Dynatech ML 3000 luminometer (Dynatech, Chantilly, VA) using cycle mode after initiating the reaction with 100 µl of Promega luciferase assay reagent. The β-galactosidase activity in 50 µl of each cell lysate was determined using a β-galactosidase assay (Promega, Madison, WI). Results were normalized by dividing the relative light units obtained for each sample by the β-galactosidase activity.

WHAT IS CLAIMED IS:

1. A method for determining if a leptin receptor binding compound is present in a sample comprising:

contacting the sample with a cell which comprises a) nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor; and

determining if transcription of the reporter gene occurs.

2. A method according to Claim 1 wherein the leptin response element is an IRF-1 derived gamma-interferon activation sequence.

3. A method according to Claim 2 wherein the IRF-1 derived gamma interferon activation sequence is CTG ATT TCC CCG AAA TGA CG.

4. A method according to Claim 3 wherein the promoter region comprises a plurality of leptin response elements.

5. A method according to Claim 4 wherein the promoter region comprises at least three IRF-1 derived gamma-interferon activated sequences.

6. A method according to Claim 3 wherein the leptin receptor is a recombinant leptin receptor.

7. A method according to Claim 6 wherein the leptin receptor is human or rodent.

8. A method according to Claim 7 wherein the nucleic acid comprising the leptin receptor comprises a sequence selected from the group of sequences consisting of those in Figures 3, 4 and 5.

9. A method according to Claim 3 wherein the transcription of the reporter gene occurs in proportion to the amount of leptin receptor binding compound present.

10. A nucleic acid comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene.

11. A nucleic acid according to Claim 10 wherein the leptin response element is CTG ATT TCC CCG AAA TGA CG.

12. A nucleic acid according to Claim 11 wherein the promoter is a minimal promoter.

13. A nucleic acid according to Claim 12 wherein the reporter gene is luciferase.

14. A vector comprising nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element, and said promoter region operatively linked to a reporter gene.

15. A set of vectors comprising

a) a first vector comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element, said promoter region operatively linked to a reporter gene; and

b) a second vector comprising nucleic acids encoding a leptin receptor.

16. A set of vectors according to Claim 15, wherein the second vector comprises a sequence selected from the group of sequences consisting of those in Figures 3, 4 and 5.

17. A cell which has been transfected with a) a vector comprising nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin-response element, and said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor.

18. A method for quantifying the amount of a leptin-receptor binding compound present in a sample comprising:

contacting the sample with a cell which has been transfected with a) nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor; and

determining the amount of transcription of the reporter gene that occurs.

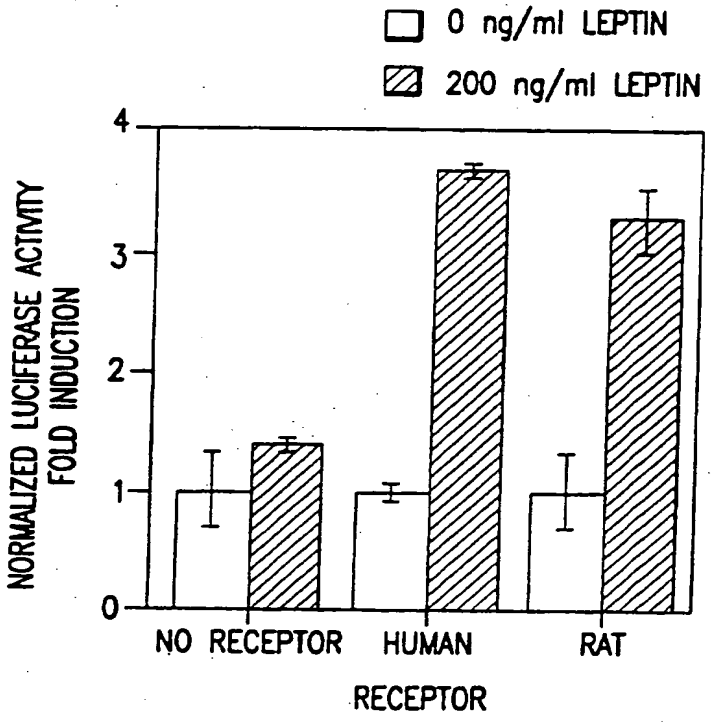
19. A method of determining if a putative leptin response element is a leptin response element comprising:

providing a cell transfected with a construct comprising a promoter region, said promoter region comprising a promoter and the putative leptin response element, said promoter region operatively linked to a reporter gene, and said cell expressing leptin receptor; and

contacting said cell with leptin.

20. A method for determining the leptin binding ability of a putative leptin receptor comprising:

providing a cell which expresses the putative leptin receptor, said cell also comprising nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element, and wherein said promoter region is operatively linked to a reporter gene; and contacting the cell with leptin.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200 ng/ml LEPTIN
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FOLD INDUCTION
4
3
2
1
0
NO RECEPTOR
HUMAN
RAT
RECEPTOR

FIG.1

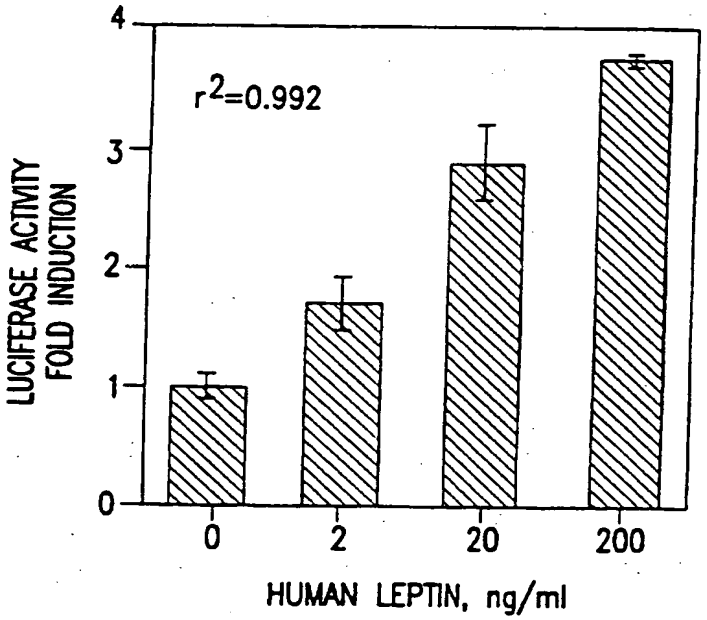
4
3
2
1
0
r^2=0.992
LUCIFERASE ACTIVITY
FOLD INDUCTION
0
2
20
200
HUMAN LEPTIN, ng/ml

FIG.2

```
   1  TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
  51  ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT
 101  TTTGTTACAC TGGGAATTTC TGTATGTGAT AACTGCACTT AACCTGGCCT
 151  ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA
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 601  CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA
 651  TGCGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
 701  GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA
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1801  GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851  TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT
1901  TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC
1951  CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCTATGAG AGGGCCTGAA
2001  TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC
```

FIG.3A

2051 CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA
2101 GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT
2151 GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC
2201 TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401 ATGATGATGA TGGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG
2451 TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT
2501 TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT
2551 TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC
2601 ATTGTACCGA TAATTATTTC CTCTTGTGTC CTGCTGCTCG GAACACTGTT
2651 AATTTCACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC
2701 CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GCCTGAAACA
2751 TTTGAGCATC TTTTTACCAA GCATGCAGAA TCAGTGATAT TTGGTCCTCT
2801 TCTTCTGGAG CCTGAACCAG TTTCAGAAGA AATCAGTGTC GATACAGCTT
2851 GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG
2901 ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTAA
2951 CAGTGCTAAC TTCTCTGGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG
3001 AGTGTCAGAG TCAACCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG
3051 AAAACAGTGG AAACTGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG
3101 CCAGTGCATC GCCAGGAAAC ATTCCCCACT GAGACAGTCT TTTTCTAGCA
3151 ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA
3201 CCCAATGTGA TTTCACCACA ACTTTCATTC TCAGGGTTGG ATGAGCTTTT
3251 GGAACTGGAG GGAAATTTTC CTGAAGAAAA TCACGGGGAA AAATCTGTGT
3301 ATTATCTAGG AGTCTCCTCA GGAAACAAAA GAGAGAATGA TATGCTTTTG
3351 ACTGATGAGG CAGGGGTATT GTGCCCATTC CCAGCTCACT GTCTGTTCAG
3401 TGACATCAGA ATCCTCCAGG AGAGTTGTTC ACACTTTGTA GAAAATAATT
3451 TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT
3501 CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAATA AGATGTGTGA
3551 CTTAACTGTG TAATCTTGTC CAAAAACTTC CAGGTTCCAT TCCAGTAGAG
3601 TGTGTCATGT ATAATATGTT CTTTTATAGT TGTGGGTGGG AGAGAAAGCC

FIG.3B

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 141  CTTATGCTG
 151  GGATGTGCCT TAGAGGATTA TGGGTGTACT TCTCTGAAGT AAGATGATTT
 201  GTCAAAAATT CTGTGTGGTT TTGTTACATT GGGAATTTAT TTATGTGATA
 251  ACTGCGTTTA ACTTGTCATA TCCAATTACT CCTTGGAGAT TTAAGTTGTC
 301  TTGCATGCCA CCAAATTCAA CCTATGACTA CTTCCTTTTG CCTGCTGGAC
 351  TCTCAAAGAA TACTTCAAAT TCGAATGGAC ATTATGAGAC AGCTGTTGAA
 401  CCTAAGTTTA ATTCAAGTGG TACTCACTTT TCTAACTTAT CCAAAACAAC
 451  TTTCCACTGT TGCTTTCGGA GTGAGCAAGA TAGAAACTGC TCCTTATGTG
 501  CAGACAACAT TGAAGGAAAG ACATTTGTTT CAACAGTAAA TTCTTTAGTT
 551  TTTCAACAAA TAGATGCAAA CTGGAACATA CAGTGCTGGC TAAAAGGAGA
 601  CTTAAAATTA TTCATCTGTT ATGTGGAGTC ATTATTTAAG AATCTATTCA
 651  GGAATTATAA CTATAAGGTC CATCTTTTAT ATGTTCTGCC TGAAGTGTTA
 701  GAAGATTCAC CTCTGGTTCC CCAAAAAGGC AGTTTTCAGA TGGTTCACTG
 751  CAATTGCAGT GTTCATGAAT GTTGTGAATG TCTTGTGCCT GTGCCAACAG
 801  CCAAACTCAA CGACACTCTC CTTATGTGTT TGAAAATCAC ATCTGGTGGA
 851  GTAATTTTCC AGTCACCTCT AATGTCAGTT CAGCCCATAA ATATGGTGAA
 901  GCCTGATCCA CCATTAGGTT TGCATATGGA AATCACAGAT GATGGTAATT
 951  TAAAGATTTC TTGGTCCAGC CCACCATTGG TACCATTTCC ACTTCAATAT
1001  CAAGTGAAAT ATTCAGAGAA TTCTACAACA GTTATCAGAG AAGCTGACAA
1051  GATTGTCTCA GCTACATCCC TGCTAGTAGA CAGTATACTT CCTGGGTCTT
1101  CGTATGAGGT TCAGGTGAGG GGCAAGAGAC TGGATGGCCC AGGAATCTGG
1151  AGTGACTGGA GTACTCCTCG TGTCTTTACC ACACAAGATG TCATATACTT
1201  TCCACCTAAA ATTCTGACAA GTGTTGGGTC TAATGTTTCT TTTCACTGCA
1251  TCTATAAGAA GGAAAACAAG ATTGTTCCCT CAAAAGAGAT TGTTTGGTGG
1301  ATGAATTTAG CTGAGAAAAT TCCTCAAAGC CAGTATGATG TTGTGAGTGA
1351  TCATGTTAGC AAAGTTACTT TTTTCAATCT GAATGAAACC AAACCTCGAG
1401  GAAAGTTTAC CTATGATGCA GTGTACTGCT GCAATGAACA TGAATGCCAT
1451  CATCGCTATG CTGAATTATA TGTGATTGAT GTCAATATCA ATATCTCATG
1501  TGAAACTGAT GGGTACTTAA CTAAAATGAC TTGCAGATGG TCAACCAGTA
1551  CAATCCAGTC ACTTGCGGAA AGCACTTTGC AATTGAGGTA TCATAGGAGC
1601  AGCCTTTACT GTTCTGATAT TCCATCTATT CATCCCATAT CTGAGCCCAA
1651  AGATTGCTAT TTGCAGAGTG ATGGTTTTTA TGAATGCATT TTCCAGCCAA
1701  TCTTCCTATT ATCTGGCTAC ACAATGTGGA TTAGGATCAA TCACTCTCTA
1751  GGTTCACTTG ACTCTCCACC AACATGTGTC CTTCCTGATT CTGTGGTGAA
1801  GCCACTGCCT CCATCCAGTG TGAAAGCAGA AATTACTATA AACATTGGAT
1851  TATTGAAAAT ATCTTGGGAA AAGCCAGTCT TTCCAGAGAA TAACCTTCAA
1901  TTCCAGATTC GCTATGGTTT AAGTGGAAAA GAAGTACAAT GGAAGATGTA
1951  TGAGGTTTAT GATGCAAAAT CAAAATCTGT CAGTCTCCCA GTTCCAGACT
2001  TGTGTGCAGT CTATGCTGTT CAGGTGCGCT GTAAGAGGCT AGATGGACTG
2051  GGATATTGGA GTAATTGGAG CAATCCAGCC TACACAGTTG TCATGGATAT
2101  AAAAGTTCCT ATGAGAGGAC CTGAATTTTG GAGAATAATT AATGGAGATA
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FIG.4A

```
2151  CTATGAAAAA GGAGAAAAAT GTCACTTTAC TTTGGAAGCC CCTGATGAAA
2201  AATGACTCAT TGTGCAGTGT TCAGAGATAT GTGATAAACC ATCATACTTC
2251  CTGCAATGGA ACATGGTCAG AAGATGTGGG AAATCACACG AAATTCACTT
2301  TCCTGTGGAC AGAGCAAGCA CATACTGTTA CGGTTCTGGC CATCAATTCA
2351  ATTGGTGCTT CTGTTGCAAA TTTTAATTTA ACCTTTTCAT GGCCTATGAG
2401  CAAAGTAAAT ATCGTGCAGT CACTCAGTGC TTATCCTTTA AACAGCAGTT
2451  GTGTGATTGT TTCCTGGATA CTATCACCCA GTGATTACAA GCTAATGTAT
2501  TTTATTATTG AGTGGAAAAA TCTTAATGAA GATGGTGAAA TAAAATGGCT
2551  TAGAATCTCT TCATCTGTTA AGAAGTATTA TATCCATGAT CATTTTATCC
2601  CCATTGAGAA GTACCAGTTC AGTCTTTACC CAATATTTAT GGAAGGAGTG
2651  GGAAAACCAA AGATAATTAA TAGTTTCACT CAAGATGATA TTGAAAAACA
2701  CCAGAGTGAT GCAGGTTTAT ATGTAATTGT GCCAGTAATT ATTTCCTCTT
2751  CCATCTTATT GCTTGGAACA TTATTAATAT CACACCAAAG AATGAAAAAG
2801  CTATTTTGGG AAGATGTTCC GAACCCCAAG AATTGTTCCT GGGCACAAGG
2851  ACTTAATTTT CAGAAGCCAG AAACGTTTGA GCATCTTTTT ATCAAGCATA
2901  CAGCATCAGT GACATGTGGT CCTCTTCTTT TGGAGCCTGA AACAATTTCA
2951  GAAGATATCA GTGTTGATAC ATCATGGAAA AATAAAGATG AGATGATGCC
3001  AACAACTGTG GTCTCTCTAC TTTCAACAAC AGATCTTGAA AAGGGTTCTG
3051  TTTGTATTAG TGACCAGTTC AACAGTGTTA ACTTCTCTGA GGCTGAGGGT
3101  ACTGAGGTAA CCTATGAGGC CGAAAGCCAG AGACAACCCT TTGTTAAATA
3151  CGCCACGCTG ATCAGCAACT CTAAACCAAG TGAAACTGGT GAAGAACAAG
3201  GGCTTATAAA TAGTTCAGTC ACCAAGTGCT TCTCTAGCAA AAATTCTCCG
3251  TTGAAGGATT CTTTCTCTAA TAGCTCATGG GAGATAGAGG CCCAGGCATT
3301  TTTTATATTA TCAGATCAGC ATCCCAACAT AATTTCACCA CACCTCACAT
3351  TCTCAGAAGG ATTGGATGAA CTTTTGAAAT TGGAGGGAAA TTTCCCTGAA
3401  GAAAATAATG ATAAAAAGTC TATCTATTAT TTAGGGGTCA CCTCAATCAA
3451  AAAGAGAGAG AGTGGTGTGC TTTTGACTGA CAAGTCAAGG GTATCGTGCC
3501  CATTCCCAGC CCCCTGTTTA TTCACGGACA TCAGAGTTCT CCAGGACAGT
3551  TGCTCACACT TTGTAGAAAA TAATATCAAC TTAGGAACTT CTAGTAAGAA
3601  GACTTTTGCA TCTTACATGC CTCAATTCCA AACTTGTTCT ACTCAGACTC
3651  ATAAGATCAT GGAAAACAAG ATGTGTGACC TAACTGTGTA ATTTCACTGA
3701  AGAAACCTTC AGATTTGTGT TATAATGGGT AATATAAAGT GTAATAGATT
3751  ATAGTTGTGG GTGGGAGAGA
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FIG.4B

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   1  TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
  51  ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT
 101  TTTGTTACAC TGGGAATTTC TGTATGTGAT AACTGCACTT AACCTGGCCT
 151  ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA
 201  ACTGATGACT CCTTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
 251  TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATTT AATTCAACTG
 301  GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
 351  AATGAGCAAG GTCAAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGGAA
 401  GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
 451  ACTGGGACAT AGAGTGCTGG ATGAAAGGGG ACTTGACATT ATTCATCTGT
 501  CATATGGAAC CATTACTTAA GAACCCCTTC AAGAATTATG ACTCTAAGGT
 551  TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTTG CCTCTGCCCC
 601  CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA
 651  TGCGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
 701  GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA
 751  TGTCACTGCA GCCCATGCTT GTTGTGAAGC CTGGAGTTTA CCTCAACTCT
 801  CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTCAT GGGACAGCCA
 851  AACAAAAGCA CCATTTCCAC TTCAATATCC GGTGAAATAT TTAGAGAATT
 901  CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
 951  GTAGACAGCG TGCTTCCTGG CTGTTCATAC GAGGTCCAGG TGAGGAGCAA
1001  GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTTA CCTCAACTCT
1051  TTACCACACA AGATGTCATG TATTTTCCAC CCAAAATTCT GACGAGTGTT
1101  GGATCCAATG CTTCCTTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151  CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201  AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTTCTCC
1251  AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA
1301  CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA
1351  TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACTAAA
1401  ATGACTTGCA GATGGTCACC CAGCACAATC CAATCACTAG TGGGAAGCAC
1451  TGTGCAGTTG AGGTATCACA GGCGCAGCCT GTACTGTCCC GATAATCCAT
1501  CTATTCGTCC TACATCAGAG CTCAAAAACT GCGTCTTACA GACAGATGGC
1551  TTTTATGAAT GTGTTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT
1601  GTGGATCAGG ATCAACCATT CTTTAGGTTC ACTTGACTCT CCACCAACGT
1651  GTGTCCTTCC TGACTCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA
1701  GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC
1751  AGTCTTTCCA GAGAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG
1801  GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851  TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT
1901  TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC
1951  CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCTATGAG AGGGCCTGAA
2001  TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC
2051  CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA
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FIG.5A

```
2101  GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT
2151  GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC
2201  TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251  ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301  AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351  ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401  ATGATGATGA TGGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG
2451  TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT
2501  TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT
2551  TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC
2601  ATTGTACCGA TAATTATTTC CTCTTGTGTC CTGCTGCTCG GAACACTGTT
2651  AATTTCACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC
2701  CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GCCTGAAACA
2751  TTTGAGCATC TTTTTACCAA GCATGCAGAA TCAGTGATAT TTGGTCCTCT
2801  TCTTCTGGAG CCTGAACCAG TTTCAGAAGA AATCAGTGTC GATACAGCTT
2851  GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG
2901  ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTAA
2951  CAGTGCTAAC TTCTCTGGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG
3001  AGTGTCAGAG TCAACCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG
3051  AAAACAGTGG AAACTGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG
3101  CCAGTGCATC GCCAGGAAAC ATTCCCCACT GAGACAGTCT TTTTCTAGCA
3151  ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA
3201  CCCAATGTGA TTTCACCACA ACTTTCATTC TCAGGGTTGG ATGAGCTTTT
3251  GGAACTGGAG GGAAATTTTC CTGAAGAAAA TCACGGGGAA AAATCTGTGT
3301  ATTATCTAGG AGTCTCCTCA GGAAACAAAA GAGAGAATGA TATGCTTTTG
3351  ACTGATGAGG CAGGGGTATT GTGCCCATTC CCAGCTCACT GTCTGTTCAG
3401  TGACATCAGA ATCCTCCAGG AGAGTTGTTC ACACTTTGTA GAAAATAATT
3451  TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT
3501  CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAATA AGATGTGTGA
3551  CTTAACTGTG TAATCTTGTC CAAAAACTTC CAGGTTCCAT TCCAGTAGAG
3601  TGTGTCATGT ATAATATGTT CTTTTATAGT TGTGGGTGGG AGAGAAAGCC
```

FIG.5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06505

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 33/53; C12N 1/20, 15/00, 15/12, 15/19
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/7.1, 7.2, 7.21, 7.32, 7.8, 320.1, 252.3, 325, 335, 336; 536/24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS; ONLINE, DIALOG, MEDLINE; search terms ob/leptin receptor and promoter, binding assay/compounds

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TARTAGLIA et al. Identification and expression cloning of a leptin receptor, OB-R. Cell. 29 December 1995, Vol. 83, pages 1263-1271, see entire document.	1-20
X	LEE et al. Abnormal splicing of the leptin receptor in *diabetic* mice. Nature. 15 February 1996, Vol. 379, pages 332-335, see entire document.	1-20
X	CHEN et al. Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in *db/db* mice. Cell. 09 February 1996, Vol. 84, pages 491-495, see entire document.	1-20
X, E	US, 5,643,748 A (SNODGRASS ET AL) 01 July 1997 (01.07.97), see entire document.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 SEPTEMBER 1997	30 SEP 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GARNETTE D. DRAPER Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06505

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	CIOFFI et al. Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. Nature Medicine. May 1996, Vol. 2, No. 5, pages 585-589, see entire document.	1-20
X, P	WANG et al. A novel leptin receptor isoform in rat. FEBS Letters. August 1996, Vol. 392, pages 87-90, see entire document.	1-20
X, P	IIDA et al. Phenotype-linked amino acid alteration in leptin receptor cDNA from zucker fatty (*fa/fa*) rat. Biochem. Biophys. Res. Comm. May 1996, Vol. 222, pages 19-26, see entire document.	1-20
X	PINE et al. Tyrosine phosphorylated p91 binds to a single element in the ISGF2/IF-1 promoter to mediate induction by IFNα and IFNγ, and is likely to autoregulate the p91 gene. The EMBO J. 1994, Vol. 13, No. 1, pages 158-167, see entire document.	1-20
X	WO 92/02639 A1 (THE SALK INSTITUTE BIOTECHNOLOGY/ INDUSTRY ASSOCIATES, INC.) 20 February 1992 (20.02.92), see the entire document, especially the claims.	1-20
X	HODGSON, J. Receptor screening and the search for new pharmaceuticals. Bio/Technology. September 1992, Vol. 10, pages 973-977, see entire document.	1-20
X, P	ROSENBLUM et al. Functional STAT 1 and 3 signaling by the leptin receptor (OB-R); reduced expression of the rat *fatty* leptin receptor in transfected cells. Endocrinology. November 1996, Vol. 137, No. 11, pages 5178-5181, see entire document.	1-20
X, P	GHILARDI et al. Defective STAT signaling by the leptin receptor in *diabetic* mice. Proc. Natl. Acad. Sci. USA. June 1996, Vol. 93, pages 6231-6235, see entire document.	1-20
X, P	BAUMANN et al. The full-length leptin receptor has signalling capabilities of interleukin 6-type cytokine receptors. Proc. Natl. Acad. Sci. USA. August 1996, Vol. 93, pages 8374-8378, see entire document.	1-20
X, P	WO 96/38586 A1 (SMITHKLINE BEECHAM PLC) 05 December 1996 (05.12.96), see entire document.	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06505

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.1, 7.2, 7.21, 7.32, 7.8, 320.1, 252.3, 325, 335, 336; 536/24.1